4

Characterization of Amphoteric Surfactants

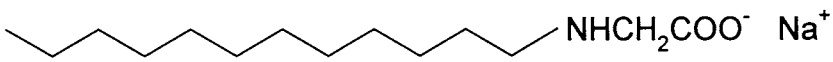
I. DESCRIPTION AND TYPICAL SPECIFICATIONS

Amphoteric surfactants can function either as anionic or cationic surfactants, depending on the pH of the system. They contain both anionic and cationic functions in the same molecule. More costly to produce than ionic surfactants, amphoteric surfactants represent only about 3% of surfactant volume in Europe and less than 1% in the United States. They are less irritating than other materials and are largely used in personal care products. A distinction can be made between amphoteric and zwitterionic surfactants. This distinction does not affect their analysis. For the analyst, a more important distinction is between amphoterics with a secondary or tertiary amine group and those containing a quaternary amine function. The former only have cationic properties when protonated at low pH, while the quaternary amines have cationic properties even under alkaline conditions.

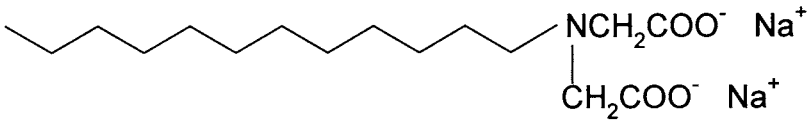
A very thorough discussion of these compounds comprises a volume of the Surfactant Science Series (1). We adopt the organization and terminology of Lomax (1) and the names used by the CTFA (2).

A. Alkylamino Acids

1. Description



N-Dodecylaminoacetic acid, sodium salt



N-Dodecyliminodiacetic acid, disodium salt

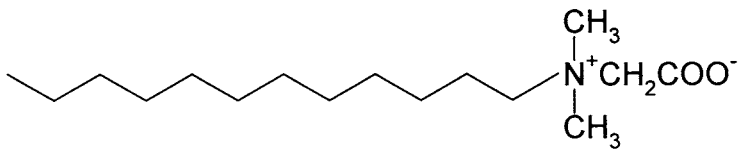
These amphoteric surfactants may be prepared by reaction of a fatty primary amine with chloroacetic acid. They are also produced by reaction of formaldehyde and HCN with a fatty amine. *N*-alkyl-β-aminopropionates and *N*-alkyl-β-iminodipropionates are usually made by reaction of the alkylamine with methyl acrylate, followed by hydrolysis of the methyl esters (3).

2. Typical Specifications

Parameter	Test method
Active agent	Alcohol-soluble portion
Free amines	Gas chromatography
Di- or tri-acids	Gas chromatography of methyl esters
NaCl and Na_2SO_4	Titration or ion chromatography
Methanol	Gas chromatography

B. Alkylbetaines

1. Description



Dodecyldimethylammononiomethane carboxylate

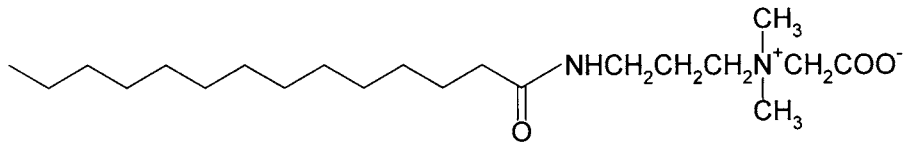
Although sulfur- and phosphorus-containing betaine derivatives are effective as amphoteric surfactants, only the carboxybetaines are widely used. They are internal salts. [Betaine is trimethylammonium acetate, $(CH_3)_3N^+CH_2COO^-$]. The most frequently encountered example is (alkyldimethylammonio)methane carboxylate (DAMC). These are most often made by quaternization of a tertiary amine with chloroacetic acid. Commercial products are generally mixtures of homologs with alkyl chain lengths in the C_8–C_{18} range (4).

2. Typical Specifications

Parameter	Test method
Assay	Titration
Sodium chloride; sodium glycolate; sodium chloroacetate	Ion chromatography
Free tertiary amine	Titration

C. Alkylamidobetaines

1. Description



A cocoamidopropylbetaine component

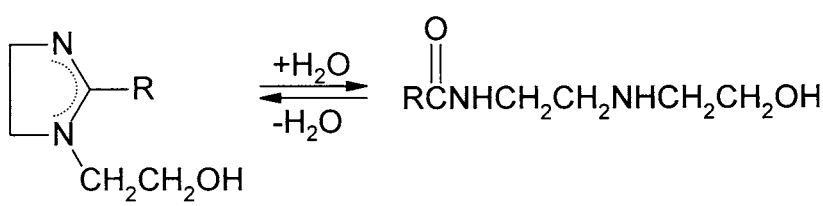
Amidobetaines are the most commercially important betaines. They tend to be high foaming, have good detergent properties, and are outstanding in their lack of irritation to skin and eyes. They are produced in a way analogous to alkylbetaines, first by reaction of a primary/tertiary diamine, such as dimethylaminopropylamine, with a fatty acid (either free fatty acid or a fatty acid methyl ester or a triglyceride) to form an alkylamidodialkylamine, then by quaternization with chloroacetic acid.

2. Typical Specifications

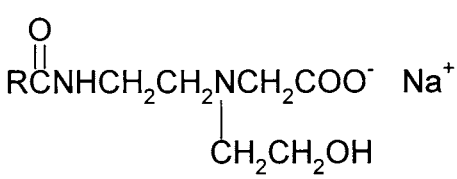
Parameter	Test method
Assay	Titration
Identity	IR
Solids content	Oven weight loss
Total N	Kjeldahl nitrogen
Glycerin	GC or HPLC
Free fatty acid content	GC
Sodium chloride; sodium glycolate; sodium chloroacetate; sodium dichloroacetate	Ion chromatography
Other tests	Saponification number; acid number; pH, 5% aqueous; color

D. Imidazoline-Derived Amphoterics

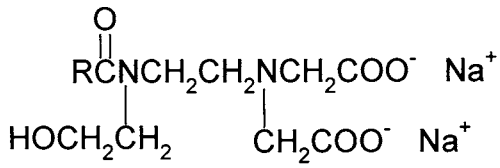
1. Description



1-Hydroxyethyl-2-alkyl-imidazoline An alkyl amidoamine



Monocarboxylated imidazoline derivative (CTFA cocoamphoacetate)



Dicarboxylated imidazoline derivative (CTFA cocoamphocarboxyglycinate)

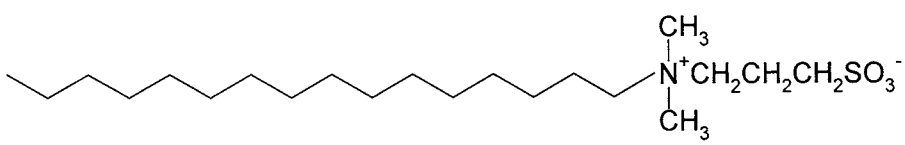
In former times it was thought that imidazole-derived amphoterics had betaine structure, but they are now classified as alkylamidoamino acids. Imidazolines are formed by condensation of a fatty acid (introduced as the free acid or as its ester with glycerin or methanol) with aminoethylethanolamine ($NH_2C_2H_4NHC_2H_4OH$), or aminopropylethanolamine. Other amines may be used, such as ethylenediamine or diethylenetriamine. Heating of the amide intermediate under vacuum results in formation of the imidazoline ring structure. The imidazoline is reacted further to add functional groups and it is during this functionalization reaction that the imidazoline structure is lost again by hydrolysis. Usually this reaction is with sodium chloroacetate. The hydrolysis product of 1-hydroxyethyl-2-alkylimidazoline, shown upper left, is the parent amidoamine, above right. A typical commercial product is the carboxylated product pictured above, with the dicarboxylate present as a trace impurity. This is true whether the product is described as being monocarboxylated or dicarboxylated (1,5,6).

2. Typical Specifications

Parameter	Test method
Assay	Titration
Identity	IR
Solids content	Oven weight loss
Total N	Kjeldahl nitrogen
Glycerin	GC or HPLC
Free fatty acid content	GC
Piperazine	GC
Sodium chloride; sodium glycolate; sodium chloroacetate; sodium dichloroacetate	Ion chromatography
Other tests	Saponification number; acid number; pH, 5% aqueous; color

E. Sulfur-Containing Amphoterics

1. Description



N-Cetyl-*N*,*N*-dimethylammoniumpropanesultaine; 3-(hexadecyldimethylammonio)-1-propanesulfonate (a component of CTFA coco-sultaine)

Sulfobetaines are the most common in this category, but even they are not widely used. Sulfobetaines are insensitive to water hardness and have excellent solubility in electrolyte

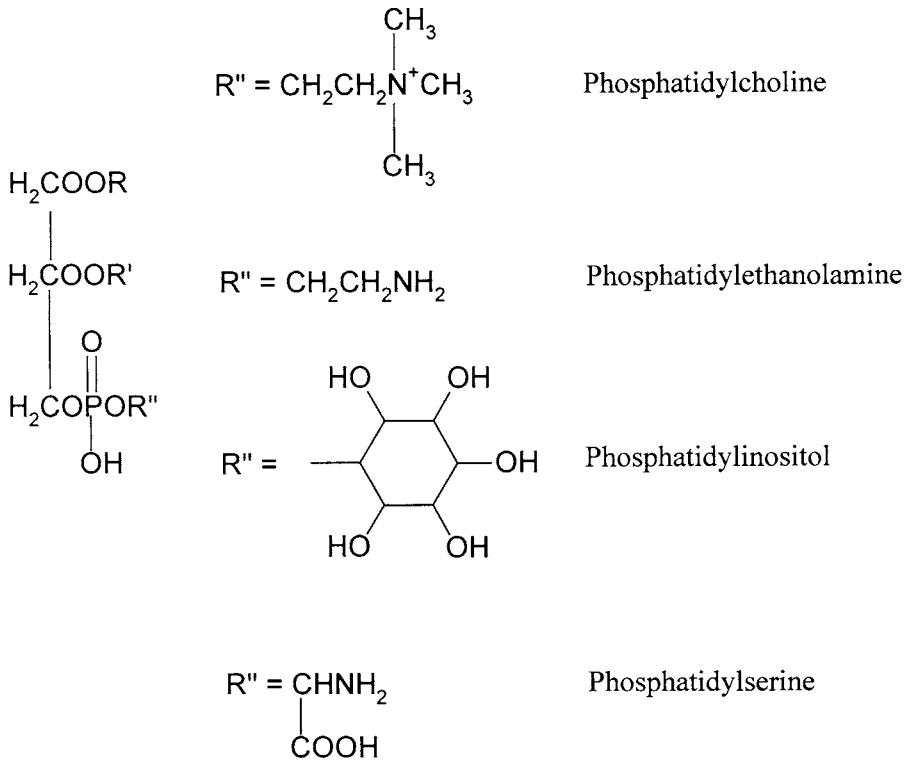
solutions. They are good foamers and also function as lime soap dispersing agents, thus synergistically increasing the detergency obtained with soap formulations. They also are effective antistats. For laundry applications, a lower wash temperature can be used with sulfobetaine surfactants than is generally effective with other surfactants.

2. Typical Specifications

Parameter	Test method
Alkyl chain length	HPLC
Free tertiary amine	HPLC
Unsulfonated quat intermediate	HPLC
Other tests	Sulfate; bisulfite; chloride; apparent pH

F. Lecithin

1. Description



Lecithin is a phosphoglyceride (phosphatide) natural product isolated commercially from soybean oil and, in much lower quantity, from egg yolks. The terminology is somewhat confused for historical reasons (7). While the term lecithin is sometimes used synonymously with α-phosphatidyl choline, it more correctly denotes a crude mixture also containing β-phosphatidyl choline and phosphatidyl esters of other compounds, chiefly ethanolamine, inositol, and serine. (While phosphatidyl choline and phosphatidyl ethanolamine are amphoteric, phosphatidyl inositol and phosphatidyl serine are anionic.) For soybean lecithin, R and R′ represent C_{16} saturated fatty acid and C_{18} saturated and unsaturated

fatty acids. Egg lecithin also contains C_{20} unsaturated fatty acid. Soybean lecithin usually contains a substantial amount of soybean oil, including free fatty acids and carbohydrates.

Lecithin is widely used as an emulsifier in foods and pharmaceuticals, as well as in coatings, printing inks, and other products. Pharmaceutical compendia in various countries give specifications for purified lecithin, generally not in terms of chemical composition but in terms of gross parameters like ash, volatile matter, nitrogen content, and the like. There is a wide range of specially treated products described as lecithin. For example, if deoiled lecithin is treated with alcohol, one fraction will be greatly enriched in alcohol-soluble phosphatidyl choline, while the other will be enriched in alcohol-insoluble phosphatidyl inositol (8).

2. Typical Specifications

Parameter	Test method
Acetone-insoluble matter	AOCS Ja 4-46
Petroleum ether insolubles	AOCS Ja 3-55
Water	Karl Fischer titration; azeotropic distillation (AOCS Ja 2-46)
Total phosphorus	Alkaline fusion, precipitation of molybdophosphoric acid, titration (AOCS Ja 5-55)
Determination of individual phosphatides	TLC (AOCS Ja 7-86); HPLC
Free fatty acid value	Titration of acetone solubles
Other tests	Apparent pH, 1% in 30:70 ethanol/water; viscosity, Brookfield, 80°C; Gardner color; peroxide value, AOCS Cd 8-53; iodine number, AOCS Cd 1-25; acid number, AOCS Ja 6-55

II. GENERAL TEST METHODS

A. Assay

The standard two-phase titration usually applied to cationics can also be used with amphoteric surfactants if the pH is adjusted. This is discussed in chapter 16. The ethanol concentration of the aqueous phase must be controlled within narrow limits for accurate results (9).

B. Acid-Base Titration

1. General Considerations

Potentiometric titration with HCl gives a characteristic value proportional to the content of amphoteric surfactant (1,10,11). In some cases, solvent systems have been optimized so that acid-base titration is suitable for assay of the product, as described below for characterization of alkylbetaines (12).

2. Amine Value

The significance of amine value varies with the product. The procedure given in Chapter 3 for analysis of cationic surfactants is based on simple titration to a visual end point and is useful for quality control of a well-characterized material. For new products, it is preferable to conduct the analysis with a recording potentiometric titrator.

C. Iodine Value

The usual procedure for determining unsaturation is the Wijs method, described in Chapter 2. The recommended ASTM and AOCS methods are D2075 and Cd 1-25, respectively (13,14).

D. Anions and Salts

The same remarks apply as for cationic surfactants in Chapter 3 (15).

E. Alkyl Chain Length Distribution

The homolog distribution of the fatty alkyl portion of these surfactants is determined by HPLC or, after appropriate decomposition of the sample, by GC. Details of these determinations are found in Chapters 7 and 8.

F. *N*-Nitrosamines

Although amphoteric surfactants are generally free of *N*-nitrosamines, those used in cosmetics may occasionally be analyzed by the method for total nitrosamines described under characterization of nonionic surfactants (Chapter 2, Section II).

III. ANALYSIS OF INDIVIDUAL SURFACTANTS

A. Alkylbetaines

The precise characterization of commercial betaines is difficult because of the variety of similar compounds which may be present in the product (1,11). Free amine concentration is a critical parameter because of the odor it gives to the product.

Alkyldimethylbetaine and free amine can be determined by potentiometric acid-base titration (12). For titration of the betaine, the sample is dissolved in a mixture of 10:1 methyl isobutyl ketone/isopropanol to which a little HCl has been added. Three breaks are observed on titration with ethanolic KOH, corresponding respectively to excess HCl, the betaine, and combined impurities: amine, glycolic acid, and monochloracetic acid (see Fig. 1). Carbon dioxide must be excluded during the titration. Sodium chloride is insoluble in the solvent system and precipitates. The titration is very sensitive to water concentration (16)

For titration of the amine, the sample is dissolved in 50:50 isopropanol/water to which excess NaOH and a small, precisely measured, amount of tri-*n*-butylamine are added. Again, CO_2 must be excluded. Titration with aqueous HCl gives two breaks, the first corresponding to excess NaOH and the second to the free amine (see Fig. 2). The amine titration is corrected for the spiked amount; spiking allows a readily visible end point even when the sample has a low free amine content. The amine determination was subjected to a collaborative study which showed that at the 0.2–0.5% level repeatability by the same laboratory was in the range of 10%, and was 20% between laboratories (17).

B. Alkylamidobetaines

Alkyl chain length is most readily determined by HPLC, as discussed in Chapter 7. NMR analysis has been proposed for assay of betaines, using the signal at 3.3 ppm versus trimethylsilylpropionate. Trimethylsilylpropionate can be used as the internal standard for

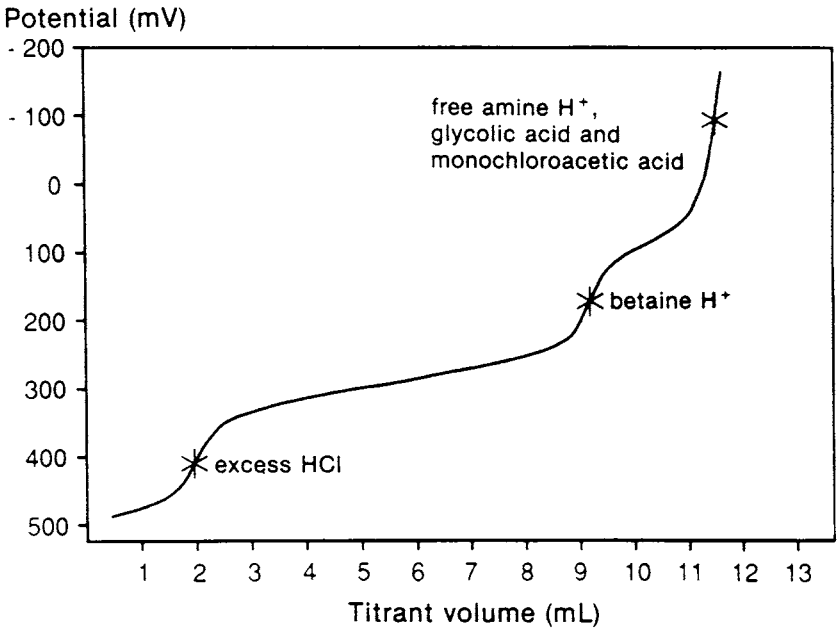


FIG. 1 Nonaqueous titration of alkyldimethylbetaine with potassium hydroxide solution. (Reprinted with permission from Ref. 12. Copyright 1993 by the American Oil Chemists' Society.)

quantification (16). Amidobetaines can be hydrolyzed with hydrochloric acid to yield the starting fatty acids.

1. Assay

These products can be determined by titration. Although they are cationic at low pH, their ion pairs with anionic surfactants are not sufficiently insoluble that the regular anionic/cationic titration method can be used. This is especially true of the lower chain length components of, for example, a cocoamidoalkylbetaine. A better system is to use tetraphenylborate ion as titrant with the electrode system typically chosen for the titration of nonionics (18).

Certain alkylamidobetaines may contain organic acids as hydrotropes (perhaps trimethylglycine or citric acid) to allow a highly concentrated product. In such cases, the active agent should be isolated by solid phase extraction prior to titration (19).

The alkylamidobetaine can be titrated in one of three ways (16,18,19):

1. Excess acid is added, and the product is titrated potentiometrically in a nonaqueous medium with alkali. The first break is due to the excess acid, the second is due to the active agent. A third break may be observed due to impurities. Acetone/2-propanol, 4:1, is a suitable solvent for use with methanolic KOH titrant. The titration is sensitive to the presence of water and CO_2.
2. Excess base is added with a little sodium acetate, and the product is titrated potentiometrically with acid in a nonaqueous system. The first break is due to excess base, the second to impurities, and the third to the active agent (Fig. 3).

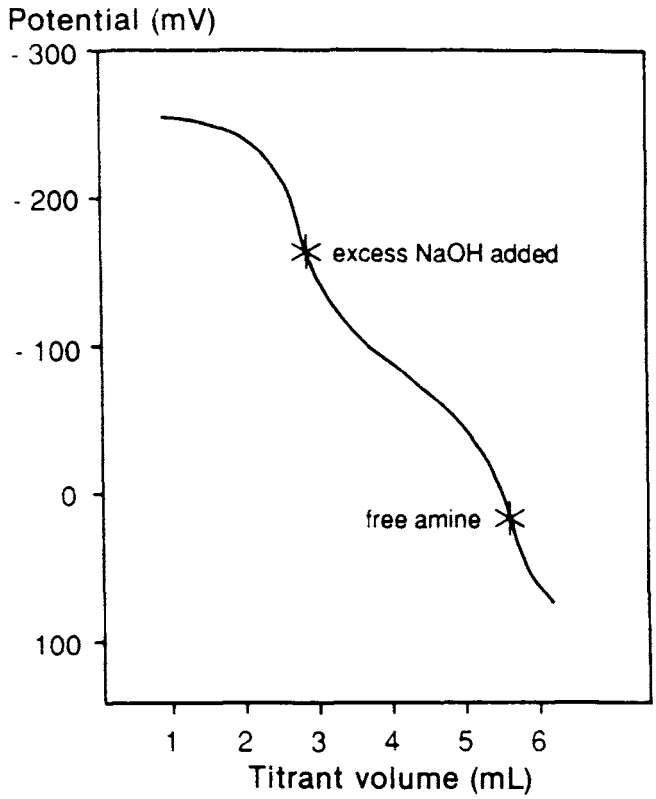


FIG. 2 Titration of free amine in alkyldimethylbetaine with hydrochloric acid. (Reprinted with permission from Ref. 12. Copyright 1993 by the American Oil Chemists' Society.)

Ethylene glycol monomethyl ether/methanol, 3:1, is a suitable solvent with perchloric acid titrant in dioxane. Other solvent systems may be used, but then citric acid (sometimes added as a hydrotrope) will interfere. Glycine, dimethylglycine, and trimethylglycine interfere regardless.

3. The product is acidified and titrated with sodium tetraphenylborate in aqueous solution using a surfactant-sensitive electrode or other electrode for end point detection, as described in Chapter 16. The addition of gum arabic to the titration vessel smooths the titration curve by preventing the deposition of the cationic/tetraphenylborate precipitate on the electrode.

In a comparison of the titrations, Buschmann and Wille report that the titration with alkali (after SPE purification) gives lower results than the titration with acid (again after purification). Titration with tetraphenylborate gives significantly lower results with poor repeatability (19).

Procedure: Separation of Cocoamidopropylbetaine from Hydrophilic Organic Acids and Alkylamidoamine by Solid Phase Extraction (19)

Use a reversed-phase cartridge such as Macherey-Nagel C_8, 6 g in a 15-mL cartridge. Dissolve 800 mg product in 3 mL 2:1 MeOH/H_2O and add to cartridge. Elute organic acid

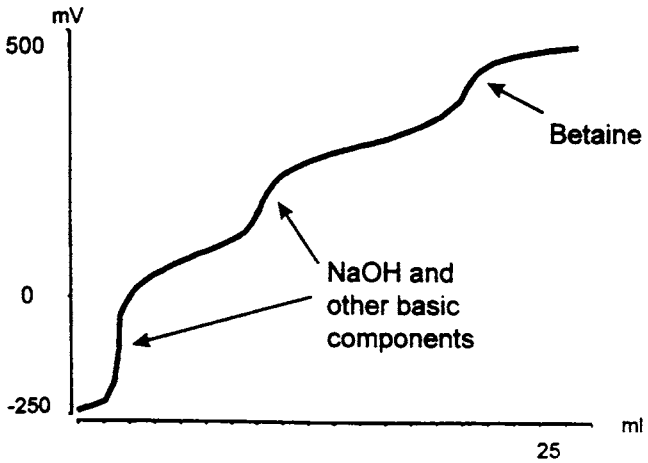


FIG. 3 Nonaqueous titration of cocoamidopropylbetaine with perchloric acid. (Reprinted with permission from Ref. 16. Copyright 1996 by Karl Hanser Verlag.)

hydrotropes with 12 mL 2:1 MeOH/H_2O, then elute the cocoamidopropylbetaine with 18 mL MeOH. Any amidoamine remains in the cartridge.

2. Determination of Unquaternized Amine

Alkylamidobetaines may contain residual unquaternized alkylamidoamine. A procedure developed specifically for determination of C_{12}–C_{18} alkylamidopropylamine in the corresponding alkylamidopropylbetaine consists of extracting the amine with hexane from a 25:75 isopropanol/0.5 M aqueous NaOH solution of the betaine. The amine is isolated from the hexane by evaporation, then determined by two-phase titration with dodecylsulfate with dimidium bromide/disulfine blue mixed indicator under acid conditions according to the method described in Chapter 16 (20). The method gives good precision for amine concentrations in the 0.3–1.0% range.

C. Imidazoline-Derived Amphoterics

Products described commercially as being monocarboxylates and dicarboxylates are produced by reaction of the imidazoline intermediate with monochloroacetic acid, 1:1 or 1:2, respectively. It has been found that the product of this reaction, the active agent, is in each case the monocarboxylate, with at most a trace of the dicarboxylate being present. The difference between the two products is that those made with an excess of monochloroacetic acid contain more of the active agent and less of the unreacted amidoamine intermediate (6).

Amphoglycinates can be determined by potentiometric titration with perchloric acid using a pH electrode or by titration with tetraphenylborate using a nonionic surfactant-selective electrode (18).

Characterization of an imidazoline-derived amphoteric requires considering the reaction pathway used to prepare the compound. For example, an amidobetaine may be made by sodium chloroacetate treatment of the imidizoline/linear amide mixture obtained by reacting a fatty acid or ester with *N*-hydroxyethylethylenediamine. Possible compo-

nents will then be free fatty acid, fatty acid esters of as many kinds as there are alcohols, free amine, free amides and diamides, amphoacetate (main component), amphodiacetate, sodium glycolate, residues from catalysts, etc. Excess sodium chloroacetate is destroyed at the end of the synthesis by hydrolysis with alkali, yielding sodium glycolate and sodium chloride. The aminoethyl ethanolamine used to prepare the intermediate amide can cyclize to form piperazine.

Schwarz and coworkers used a number of titration procedures to characterize the intermediates of the synthesis. They differentiated the content of primary, secondary, and tertiary amine as well as amide functionality (21). Hydroxyethyl groups are determined by titration with periodate ion, with a preliminary extraction step serving to separate aminoethylethanolamine from the alkylamidoethylethanolamine.

1. Chromatographic Analysis

Imidazoline derivatives can be hydrolyzed with sulfuric acid into their starting materials: diamines and fatty acids. These can then be analyzed by gas chromatography, as described in Chapter 8. Some impurities, as well as the main components, can be detected (22,23). As described in Chapter 9, TLC may be used for semiquantitative determination of various compounds: main component, imidazoline intermediate, secondary and tertiary amide intermediates, and *N*-hydroxyethylethylenediamine starting material (5). HPLC methods used to analyze imidazoline derivatives are summarized in Chapter 7. Separation is typically according to the length of the alkyl chain, with further differentiation of impurities and intermediates within each alkyl chain group (24,25). HPLC has been demonstrated for the determination of free dimethylaminopropylamine after derivatization with salicylaldehyde (26).

2. Determination of Imidazoline Structure

Amidobetaines were once thought of as containing the imidazoline structure. However, it is now known that the imidazoline ring is only formed as an intermediate structure which is hydrolyzed under the alkaline conditions of the final carboxymethylation reaction to form an amphoteric surfactant. Amidobetaines contain no more than a trace of the imidazoline ring (5,6). The imidazoline ring gives a relatively strong UV absorption at 235–244 nm. It is necessary to have standard compounds to perform a quantitative determination based on this phenomenon. The amide functionality also gives an absorbance near this region, making it necessary to compensate with an imidazoline-free amide solution of the same concentration (21,27).

D. Lecithin

Lecithin is analyzed according to the AOCS Ja method series (28). For food uses, the *Food Chemicals Codex* should also be consulted. These procedures are mainly based on gross physical properties, such as acetone solubility. An exception is the TLC method, which gives the composition in terms of individual phosphatides (29). Because of their importance in biology, phosphatides are the focus of intense method development. Mass spectrometry is the most powerful tool to characterize mixtures of phophatides (see Chapter 15), but there is usually no need to perform a complete MS analysis of lecithin. Commercial lecithin is a complex mixture, the properties of which are profoundly influenced by the considerable amount of nonphosphatide materials present. Further information on lecithin can be found in specialized publications (30).

1. Total Phospholipids

Total phospholipid content of soybean lecithin can be obtained by solid phase extraction on silica. Lipids are eluted with 20:80 hexane/ethyl ether, then phospholipids are eluted with methanol. The amount of each fraction is determined gravimetrically after solvent removal (31).

2. Phospholipid Profile

HPLC is the most common technique applied to the determination of the chemical composition of lecithin. Normal phase HPLC is convenient for the determination of the major constituents (i.e., phosphatidylcholine, phosphatidylethanolamine, etc), as described in Chapter 7. ^{31}P NMR is also suitable for this analysis, as discussed in Chapter 14. The biochemical literature contains many enzymatic methods, mainly for specific determination of phosphatidylcholine and its hydrolysis product, choline (32). For instance, phosphatidylcholine can be hydrolyzed by phospholipase C to a diacylglycerol and the phosphate ester of choline, which itself can be hydrolyzed by alkaline phosphatase to form choline and phosphate ion. Alternatively, action of phospholipase D on phosphatidylcholine yields phosphatidic acid and choline. These methods are not applied to analysis of the commercial lecithin used as a surfactant.

3. Fatty Acid Composition of the Phospholipids

In soybean lecithin, the acyl groups consist of oleic, linoleic, linolenic, stearic, and palmitic acids. Hydrogenated lecithin contains only stearic and palmitic acid groups. Lecithin can be characterized in terms of its total fatty acid composition. Total fatty acid composition is determined by saponification of the ester and esterification of the isolated acids with BF_3 in methanol, followed by gas chromatography of the fatty acid methyl esters (33). There is evidence that methanolic sodium methoxide gives the best yields in transesterification (34).

Reversed-phase HPLC is capable of separating the individual phosphatides according to the acyl substitution. Details of the HPLC analysis are found in Chapter 7. Enzymatic techniques allow selective hydrolysis of either the phosphorus-containing component or the fatty acids in the beta position, so that the resulting fragments can be analyzed by GC, GC-MS, and other methods (35–37).

REFERENCES

1. Lomax, E. G., ed., *Amphoteric Surfactants*, 2nd ed., Marcel Dekker, New York, 1996.
2. Cosmetics, Toiletries and Fragrances Association, *International Cosmetic Ingredient Dictionary and Handbook*, 7th ed., Washington, DC, 1997.
3. Rosenblatt, W., Amino acid amphoterics, in E. G. Lomax, ed., *Amphoteric Surfactants*, 2nd ed., Marcel Dekker, New York, 1996.
4. Domingo, X., Betaines, in Lomax, E. G., ed., *Amphoteric Surfactants*, 2nd ed., Marcel Dekker, New York, 1996.
5. Li, Z., Z. Zhang, Amphoteric imidazoline surfactants, *Tenside, Surfactants, Deterg.*, 1994, *31*, 128–132.
6. Derian, P. J., J. M. Ricca, F. Marcenac, R. Vukov, D. Tracy, M. Dahanayake, Imidazoline-derived amphoteric surfactants, *SÖFW Journal*, 1995, *121*, 399–410.
7. Parnham, M. J., Phospholipid terminology, *Inform*, 1996, *7*, 1168–1175.
8. Krawczyk, T., Lecithin: consider the possibilities, *Inform*, 1996, *7*, 1158–1159, 1162–1167.

9. Rosen, M. J., F. Zhao, D. S. Murphy, Two-phase mixed indicator method for the determination of zwitterionic surfactants, *J. Am. Oil Chem. Soc.*, 1987, *64*, 439–441.
10. Holzman, S., N. Avram, Amphoteric surfactants, *Tenside, Surfactants, Deterg.*, 1986, *23*, 309–313.
11. Lomax, E., Amphoteric surfactants, in M. R. Porter, ed., *Recent Developments in the Analysis of Surfactants*, Elsevier, London, 1991.
12. Plantinga, J. M., J. J. Donkerbroek, R. J. Mulder, Betaine and free amine in alkyldimethyl betaine by potentiometric titrations, *J. Am. Oil Chem. Soc.*, 1993, *70*, 97–99.
13. American Society for Testing and Materials, Iodine value of fatty amines, amidoamines, and diamines, D2075-66 (1981). West Conshohocken, PA 19428.
14. American Oil Chemists' Society, Iodine value of fats and oils, Official Method Cd 1-25, 2211 West Bradley Ave., Champaign, IL 61826.
15. Humbert, M., A. Hernandez, Monochloroacetate, dichloroacetate, and glycolate in betaines, *World Surfactants Congr., 4th*, 1996, *1*, 379–388.
16. Gerhards, R., I. Jussofie, D. Käseborn, S. Keune, R. Schulz, Cocoamidopropyl betaines, *Tenside, Surfactants, Deterg.*, 1996, *33*, 8–14.
17. Arens, M., H. P. Wingen, Alkyldimethylbetaines—Determination of free amine—collaboration of the DGF. Communication 149: German standard methods for study of fats, fatty products, surfactants and related materials. Communication 115: Analysis of surface active materials XXIV (in German), *Fett Wiss. Technol.*, 1995, *97*, 430–431.
18. Schulz, R., *Titration of Surfactants and Pharmaceuticals* (in German), Verlag für chemische Industrie, H. Ziolkowsky GmbH, Augsburg, 1996.
19. Buschmann, N., H. Wille, Titration of amphoteric surfactants—comparison of methods, *Comun. Jorn. Com. Esp. Deterg.*, 1997, *27*, 65–70.
20. Arens, M., R. Spilker, Fatty acid amidopropylbetaines—determination of fatty acid amidopropylamine—collaboration of the DGF. Communication 150: German standard methods for study of fats, fatty products, surfactants and related materials. Communication 116: Analysis of surface active materials XXV (in German), *Fett Wiss. Technol.*, 1995, *97*, 468–470.
21. Schwarz, G., P. Leenders, U. Ploog, Condensation products of fatty acids or their methyl esters with aminoethylethanolamine (in German), *Fette, Seifen, Anstrichm.*, 1979, *81*, 154–158.
22. Takano, S., K. Tsuji, Structural analysis of the amphoteric surfactants obtained by the reaction of 1-(2-hydroxyethyl)-2-alkyl-2-imidazoline with ethyl acrylate, *J. Am. Oil Chem. Soc.*, 1983, *60*, 1798–1806.
23. Takano, S., K. Tsuji, Structural analysis of the amphoteric surfactants obtained by the reaction of 1-(2-hydroxyethyl)-2-alkyl-2-imidazoline with sodium monochloroacetate, *J. Am. Oil Chem. Soc.*, 1983, *60*, 1807–1815.
24. Kawase, J., K. Tsuji, Y. Yasuda, K. Yashima, Amphoteric surfactants by LC with post-column detection. II: Imidazoline-type amphoteric surfactants derived from sodium chloroacetate, *J. Chromatogr.*, 1983, *267*, 133–148.
25. Kawase, J., K. Tsuji, Y. Yasuda, Amphoteric surfactants by LC with post-column detection. III: Salt-free-type imidazoline amphoteric surfactants, *J. Chromatogr.*, 1983, *267*, 149–166.
26. Prieto-Blanco, M. C., P. López-Mahía, D. Prada Rodríguez, Dimethylaminopropylamine in alkylaminoamides by HPLC, *J. Chromatogr. Sci.*, 1997, *35*, 265–269.
27. Bailles, F. Ch. Paquot, Synthesis of 2-alkyl-1-(hydroxyethyl)imidazolines and appropriate analytical methods (in French), *Chim. Phys. Appl. Prat. Ag. Surface, C. R. Congr. Int. Deterg., 5th*, Sept 9–13, 1968, *1*, 267–273.
28. American Oil Chemists' Society, Official and tentative methods. Section J: Analysis of lecithin. 2211 West Bradley Ave., Champaign, IL 61826.
29. Erdahl, W. L., A. Stolyhwo, O. S. Privett, Soybean lecithin by TLC and HPLC, *J. Am. Oil Chem. Soc.*, 1973, *50*, 513–515.
30. Szuhaj, B. F., ed., *Lecithins: Sources, Manufacture and Uses*, American Oil Chemists' Society, Champaign, IL, 1989.

31. Merton, S. L., Soybean lecithins and beef phospholipids by HPLC with an evaporative light scattering detector, *J. Am. Oil Chem. Soc.*, 1992, *69*, 784–788.
32. Kotsira, V. P., Y. D. Clonis, Colorimetric assay for lecithin using two co-immobilized enzymes and an indicator dye conjugate, *J. Agric. Food Chem.*, 1996, *46*, 3389–3394.
33. Dornbos, D. L., R. E. Mullen, E. G. Hammond, Phospholipids of environmentally stressed soybean seeds, *J. Am. Oil Chem. Soc.*, 1989, *66*, 1371–1373.
34. Eder, K., A. M. Reichlmayr-Lais, M. Kirchgessner, Methanolysis of small amounts of purified phospholipids for GC analysis of fatty acid methyl esters, *J. Chromatogr.*, 1992, *607*, 55–67.
35. Blank, M. L., L. J. Nutter, O. S. Privett, Structure of lecithins, *Lipids*, 1966, *1*, 132–135.
36. Rezanka, T., M. Podojil, Separation of algal polar lipids and of individual molecular species by HPLC and their identification by GC-MS, *J. Chromatogr.*, 1989, *463*, 397–408.
37. Ohshima, T., C. Koizumi, Selected ion monitoring GC-MS of 1,2-diacylglycerol *tert*-butyldimethylsilyl ethers derived from glycerophospholipids, *Lipids*, 1991, *26*, 940–947.